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Electron Microscopic Study on Selected Fungal Infected Organs of Rainbow Trout and Indian Hill Trout Scanning from Central Himalaya

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Abstract: *In the present study, fungal infected and healthy adult rainbow trout (Oncorhynchus mykiss) and Indian hill trout (Barilius bendelisis) were examined under scanning electron microscope to investigate the pathological alterations in gill, fin, skin and eye. The gill lamellae of infected rainbow trout were found ruptured and showed structural disorganization with fusion of respiratory lamellae and reduced interlamellar space. The eyes were found to be completely covered with mycelium. Descaling, scale erosion, abrasion of the skin and sloughing off epithelium was noticed in infected fish. The fins were found to be invaded by fungal hyphae and mycelium covered the fin epidermis. Moreover, the epithelial structure of fins was lost. In conclusion, fungi primarily infected all the boundary tissues which resulted in pathological alterations and eventual mortalities.*

Keywords: Rainbow trout, Hill trout, SEM, Fungi, Cold water.

Introduction

Disease outbreaks are the major constraint in the development and sustainability of aquaculture industry as it results in disastrous economic losses to the farmers. Fungal infections are second only to bacterial diseases in economic importance and it normally lead to chronic and steady losses (Bruno and Wood, 1994). One characteristic of fungal infections is the sporadic nature of outbreaks. Fungi are generally opportunistic pathogens that can create problems in stressed, emaciated, physically injured and diseased fish (Roth, 1972; Piper *et al*., 1983). Poor water quality can also lead to an increase in fungal infections in fish. Fungal infections are widespread among fish species and can be lethal if not treated at appropriate time. Primarily, fungi appear as white thread like structures that quickly grow into a cottony mass on the body surface and finally infiltrate into the organs and deeper tissues leading to mass mortalities (Piper *et al*., 1983). Each growing colony bears numerous sporangia, releasing large numbers of motile zoospores, the main dispersive and infective agents in the fungal life cycle.

Rainbow trout (*Oncorhynchus mykiss*) and hill trout (*Barilius bendelesis*) are highly valued food fishes that are commercially cultured in the coldwater regions of the country. As with other fishes, disease outbreak is one of the major menace threatening coldwater fisheries and aquaculture. Studies on scanning electron microscopic examinations on the fungal infected tissues particularly in coldwater fish are limited to the best of our knowledge. Skin, fins and gills constitute the boundary tissue of the fish, and, being continuously hydrated is more susceptible to various infectious agents. Therefore, the present study was aimed to investigate the pathological alterations in the gill, eye, skin and fin of fungal infected rainbow trout and Indian hill trout by scanning electron microscopy which will result in a better understanding of fish mycosis particularly their route of invasion.

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Materials and Methods

Sample collection

Fungal infected and healthy adult rainbow trout (*Oncorhynchus mykiss*) and Indian hill trout (*Barilius bendelisis)* were collected from Experiment Field Centre of ICAR- Directorate of Coldwater Fisheries Research (DCFR), Champawat (80° 07' N, 29° 30' N and an altitude of 1620 msl), Uttarakhand, India and fish culture ponds of DCFR Bhimtal, Nainital, Uttarakhand, India respectively. After, being taken to the laboratory, fishes were dissected and the samples of gill, skin, fin and eye were collected for scanning electron microscopy.

Scanning Electron Microscopy (SEM)

Different tissues were fixed in Karnovsky's fixative (containing 2.5% glutaraldehyde and 2.5% paraformaldehyde prepared in 0.1 M sodium phosphate buffer of pH 7.4) for 6-12 hours and kept at 4° C. The tissues were washed thrice thoroughly in 0.1 M sodium cacodylate buffer (pH 7.4) for fifteen minutes each at 4° C. Then, they were dehydrated twice using graded concentrations of acetone at 30, 50, 70, 80, 90, 95 % and 100% at 4° C for fifteen minutes each. The samples were dried to the critical point (i.e. 35° C at 1100 p.s.i.) in a liquid CO_2 drier (BALTEC). Thereafter, they were mounted on brass stubs, metallized with gold-palladium ions in Desk II Denton Vacuum equipment and observed under a scanning electron microscope (JEOL-JSM 6610 LV).

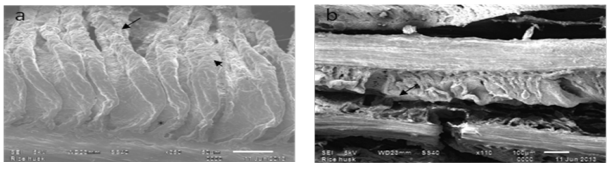

Fig. 1 SEM images of fungal infected rainbow trout gill lamellae (a) The rupture of secondary lamella (arrow), swelling at distal tip of secondary lamellae (short arrow), decreased inter lamellar space (*), (b) Fusion of secondary lamellae (arrow).

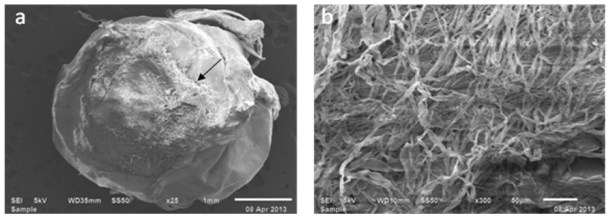

Fig. 2 SEM images of fungal infected *Barilius bendelisis* eye (a) Fungal hyphae on eye (arrow), (b) Filamentous network of hyphae.

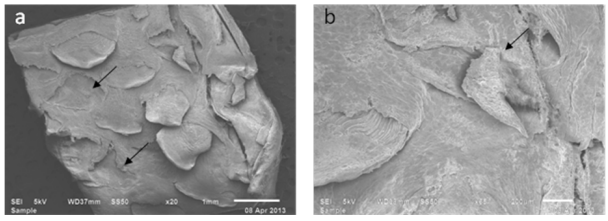

Fig. 3 SEM images of fungal infected rainbow trout skin (a) Lifting of scale (arrow), (b) Abrasion of skin (arrow).

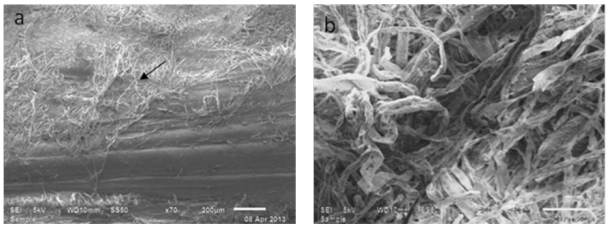

Fig. 4 SEM images of fungal infected *Barilius bendelisis* fin (a) fungal hyphae invaded the epithelium of fin, (b) Flat long hair like structure of mycelium.

Results and Discussion

Gill

In the present study, the gill lamellae in fungal infected rainbow trout were found ruptured and showed structural disorganization under scanning electron microscope. Fusion of respiratory lamellae was clearly visible. Interlamellar space was also decreased. Swelling was prominent at the distal tip of gill filament.

The gills perform homeostatic functions such as respiration, acid base balance, osmoregulation and nitrogen excretion (Thopon *et al.*, 2003;

Evans *et al.*, 2005). The basic functional unit of the gill is the filament, which supports rows of plate-like lamellae (Wilson and Laurent, 2002). Surface of gills are covered with pavement cells and the small holes in the lamellae are occupied by pillar cells (Wilson and Laurent, 2002). Gills are known to be very sensitive to different environmental conditions and can undergo pathological alterations. In the present study, decrease in the respiratory surface area by fusion of lamellae might have reduced the oxygen uptake by gills leading to hypoxia and impairment of gill function and thereby increased fish mortality.

Eye

Scanning electron microscopic examination of fungi infected eye of *Barilius bendelisis* revealed that the eye is covered with fungal hyphae which grew abundantly and formed as a filamentous network of hyphae. Similar to our observation, the eyes of fungal infected tilapia was found to be completely covered by mycelium. Fungal infections are known to cause massive destruction to fish eye impairing the vision.

Skin

In fungal infected rainbow trout, lifting and loss of scale, abrasion of the skin and disorganization of epithelium with shrunken morphology was observed at some places under scanning electron microscope. Fish skin serves as multi-functional organ playing important role in protection against injury, friction reduction (Rosen and Cornford, 1971), communication, sensory perception, locomotion (Rosen and Cornford, 1971; Long *et al*., 1996), respiration (Nonnotte, 1981), ion regulation (Handy *et al*., 1989; Fouz *et al*., 2000) and excretion (Shih *et al*., 2008). The skin is composed of three layers, an outer layer epidermis, an underlying dermis and the innermost hypodermis. Environmental stressors may induce a cellular response and cause skin damage (Udomkusonsri *et al*., 2004) and impede with its protective role (Noga, 2000). Abrasion of skin provides a portal of entry inside the body by removing epithelium including goblet cells which produce mucous the primary defense against fungus (Wood *et al*., 1988). Abnormal structure of skin led to impairment of its functional activity and affects the growth and health of fish.

Scales play important role in mechanical defense for deeper tissue and it helps in locomotion (Burdak, 1979). Several authors reported that loss of scale impaired locomotory ability of fish and provide gateway to invade fungus inside the body (Carballo and Munoz, 1991; Carballo *et al.*, 1995).

Fin

In fungal infected *Barilius bendelisis*, fins were invaded by fungal hyphae and epithelial structure was lost. Mycelium covered the epidermis of fin characterized by the presence of long, flat hairs in bundles. The growing edge of mycelium was clearly visible. The fins are folds of skin supported by skeletal rays or lepidotrichia. Fins are essential for locomotion, stabilization and also perform sensory function (Lauder and Peter, 2007; Standen, 2008). Fin damage is reported to have a negative impact on swimming performance of fish and adversely affected feeding and predator avoidance (Fu *et al.*, 2012).

Conclusion

In the present study, we closely examined the ultrastructural changes in different tissues associated with fungal infection and found that the fungi primarily infect boundary tissues like skin, eye, gills and fins resulting in pathological alterations and mortalities.

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